



Genomic DNA from blood

User manual
NucleoSpin® Blood

June 2014 / Rev. 14

MACHEREY-NAGEL
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Genomic DNA from blood

Protocol-at-a-glance (Rev. 14)

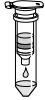
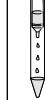
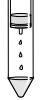
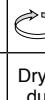
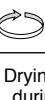
| | Mini NucleoSpin® Blood | Midi NucleoSpin® Blood L | Maxi NucleoSpin® Blood XL | Mini NucleoSpin® Blood QuickPure |
|--|---|---|---|--|
| 1 Lyse blood samples |  200 µL blood 25 µL Pro.K 200 µL B3  Mix  70 °C, 10–15 min |  2 mL blood 150 µL Pro.K 2 mL BQ1  Mix  56 °C, 10–15 min |  10 mL blood 500 µL Pro.K 10 mL BQ1  Mix  56 °C, 10–15 min |  200 µL blood 25 µL Pro.K 200 µL BQ1  Mix  70 °C, 10–15 min |
| 2 Adjust DNA binding conditions | 210 µL ethanol | 2 mL ethanol | 10 mL ethanol | 200 µL ethanol |
| 3 Bind DNA |  Load all  11,000 x g, 1 min |  Load 3 mL  4,500 x g, 3 min |  Load 15 mL  4,000 x g, 3 min |  Load all  11,000 x g, 1 min |
| | — | Load 3 mL of residue  4,500 x g, 5 min | Load 15 mL of residue  4,000 x g, 3 min | — |
| 4 Wash silica membrane |  500 µL BW 600 µL B5 1st wash  11,000 x g, 1 min |  2 mL BQ2 2 mL BQ2  4,500 x g, 2 min |  7.5 mL BQ2 7.5 mL BQ2  4,000 x g, 2 min |  350 µL BQ2  11,000 x g, 3 min |
| | 2nd wash  11,000 x g, 1 min |  4,500 x g, 10 min |  4,000 x g, 10 min | — |
| 5 Dry silica membrane |  11,000 x g, 1 min | Drying is performed during centrifugation of the last washing step | Drying is performed during centrifugation of the last washing step | Drying is performed during centrifugation of the last washing step |
| 6 Elute highly pure DNA |  100 µL BE, (70 °C)  RT, 1 min |  200 µL BE (70 °C),  RT, 2 min |  500 µL– 2000 µL BE (70 °C)  RT, 2 min |  50 µL BE (70 °C)  RT, 1 min |
| |  11,000 x g 1 min |  4,500 x g, 2 min |  4,000 x g, 2 min |  11,000 x g, 1 min |



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1 Components

1.1 Kit contents

| NucleoSpin® Blood | | | |
|---|-----------------------|-----------------------|-------------------------|
| REF | 10 preps 740951.10 | 50 preps 740951.50 | 250 preps 740951.250 |
| Buffer B3 | 10 mL | 15 mL | 60 mL |
| Wash Buffer BW | 6 mL | 30 mL | 150 mL |
| Wash Buffer B5 (Concentrate)* | 6 mL | 12 mL | 50 mL |
| Elution Buffer BE** | 13 mL | 13 mL | 60 mL |
| Proteinase K (lyophilized)* | 6 mg | 30 mg | 2 x 75 mg |
| Proteinase Buffer PB | 1.8 mL | 1.8 mL | 8 mL |
| NucleoSpin® Blood Columns (red rings – plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 20 | 100 | 500 |
| User manual | 1 | 1 | 1 |

* For preparation of working solutions and storage conditions see section 3.

**Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.1 Kit contents *continued*

| NucleoSpin® Blood L | |
|--|-----------------------|
| REF | 20 preps 740954.20 |
| Lysis Buffer BQ1 | 45 mL |
| Wash Buffer BQ2 (Concentrate)* | 20 mL |
| Elution Buffer BE** | 13 mL |
| Proteinase K (lyophilized)* | 60 mg |
| Proteinase Buffer PB | 8 mL |
| NucleoSpin® Blood L Columns (plus Collection Tubes) | 20 |
| Collection Tubes (15 mL) | 20 |
| User manual | 1 |

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.1 Kit contents *continued*

| NucleoSpin® Blood XL | | |
|---|-----------------------|-----------------------|
| REF | 10 preps 740950.10 | 50 preps 740950.50 |
| Lysis Buffer BQ1 | 125 mL | 3 x 200 mL |
| Wash Buffer BQ2 (Concentrate)* | 50 mL | 4 x 50 mL |
| Elution Buffer BE** | 30 mL | 125 mL |
| Proteinase K (lyophilized)* | 126 mg | 5 x 126 mg |
| Proteinase Buffer PB | 8 mL | 35 mL |
| NucleoSpin® Blood XL Columns (plus Collection Tubes) | 10 | 50 |
| Collection Tubes (50 mL) | 10 | 50 |
| User manual | 1 | 1 |

* For preparation of working solutions and storage conditions see section 3.

**Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.1 Kit contents *continued*

| NucleoSpin® Blood QuickPure | | | |
|---|-----------------------|-----------------------|-------------------------|
| REF | 10 preps 740569.10 | 50 preps 740569.50 | 250 preps 740569.250 |
| Lysis Buffer BQ1 | 13 mL | 13 mL | 60 mL |
| Wash Buffer BQ2 (Concentrate)* | 7 mL | 7 mL | 2 x 20 mL |
| Elution Buffer BE** | 13 mL | 13 mL | 60 mL |
| Proteinase K (lyophilized)* | 6 mg | 30 mg | 2 x 75 mg |
| Proteinase Buffer PB | 1.8 mL | 1.8 mL | 8 mL |
| NucleoSpin® Blood QuickPure Columns (dark red rings – plus Collection Tubes) | 10 | 50 | 250 |
| Collection Tubes (2 mL) | 10 | 50 | 250 |
| User manual | 1 | 1 | 1 |

* For preparation of working solutions and storage conditions see section 3.

**Composition of Elution Buffer BE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96–100 % ethanol
- Phosphate-buffered saline (PBS) may be required for some samples

Consumables

- 1.5 mL microcentrifuge tubes (NucleoSpin® Blood/QuickPure), 15 mL (NucleoSpin® Blood L), or 50 mL centrifuge tubes (NucleoSpin® Blood XL), for sample lysis and DNA elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (NucleoSpin® Blood/QuickPure), centrifuge for 15 mL (NucleoSpin® Blood L) or 50 mL (NucleoSpin® Blood XL) centrifuge tubes, with a swing-bucket rotor
- Vortex mixer
- Thermal heating block (NucleoSpin® Blood/QuickPure) or water bath (NucleoSpin® Blood L/XL)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Blood** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at www.mn-net.com.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Blood** method, genomic DNA is prepared from whole blood, cultured cells, serum, plasma, or other body fluids. Lysis is achieved by incubation of whole blood in a solution containing large amounts of chaotropic ions in the presence of Proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **NucleoSpin® Blood Columns** are achieved by addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations. With the **NucleoSpin® Blood QuickPure** kit, contaminations are removed by a single wash step. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

2.2 Kit specifications

- The **NucleoSpin® Blood** kits are designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids. It is also possible to purify viral DNA (e.g., HBV) from blood samples. As viral DNA copurifies with cellular DNA, we recommend using cell-free samples (serum or plasma) to prepare pure viral DNA.
- The **NucleoSpin® Blood QuickPure** kit is designed for ultra-fast small-scale purification of highly pure genomic DNA from whole blood, serum, plasma, or other body fluids. The number of washing and drying steps is reduced from 3 to 1! Therefore, the hands-on time is less than 10 min.
- DNA can be purified successfully from blood samples treated with EDTA, citrate, or heparin. If leukocyte rich materials like buffy coat are used, apply smaller volumes and dilute the samples with sterile PBS (dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄, and 0.24 g KH₂PO₄ in 800 mL H₂O. Adjust pH to 7.4 with HCl. Add H₂O to 1 liter).
- The kits allow purification of highly pure genomic DNA with an A₂₆₀/A₂₈₀ ratio between 1.60 and 1.90 and a typical concentration of 40–60 ng per µL for the **NucleoSpin® Blood** kit, 80–120 ng per µL for the **NucleoSpin® Blood QuickPure** kit and 200–300 ng per µL for the **NucleoSpin® Blood L/XL** kits.
- The obtained DNA is ready-to-use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.

Table 1: Kit specifications at a glance

| Parameter | Blood | Blood L | Blood XL | Blood QuickPure |
|------------------|---|---|--|---|
| Sample material | Up to 200 µL / 5 x 10 ⁶ cells | Up to 2 mL / 2 x 10 ⁷ cells | Up to 10 mL / 1 x 10 ⁸ cells | Up to 200 µL / 5 x 10 ⁶ cells |
| Typical yield | 4–6 µg | 40–60 µg | 200–300 µg | 4–6 µg |
| Elution volume | 60–200 µL | 120–200 µL | 600–2000 µL | 30–50 µL |
| Binding capacity | 60 µg | 250 µg | 700 µg | 50 µg |
| Preparation time | 30 min/prep | 1 h/prep | 1 h/prep | < 10 min/prep |
| Format | Mini spin column | Midi spin column | Maxi spin column | Mini spin column |

2.3 Storage of blood samples

For the isolation of genomic DNA from blood treated with anticoagulants (heparin, citrate, or EDTA) using a **NucleoSpin® Blood** kit the blood samples can be stored at room temperature, +4 °C, or frozen.

Blood samples stored at room temperature or +4 °C for up to several days or weeks, respectively, will still allow DNA isolation. However, DNA yield and quality will slowly decrease due to prolonged storage of blood samples under these conditions.

Blood stored frozen for years is well suited for DNA isolation.

Highest yields and quality of DNA are obtained from fresh blood.

2.4 Elution procedures

It is possible to adapt elution method and volume of elution buffer to the subsequent application of interest. In addition to the standard method (recovery rate about 70–90 %) there are several modifications possible. Use elution buffer preheated to 70 °C for one of the following procedures:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid can be eluted.
- **High concentration:** Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be higher than with standard elution (**NucleoSpin® Blood**: ca. 130 %; **NucleoSpin® Blood QuickPure**: ca. 150 ; **NucleoSpin® Blood L**: ca. 140 %; **NucleoSpin® Blood XL**: ca. 115 %). Maximal yield of bound nucleic acid is about 80 %.

- **High yield and high concentration:** Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 85–100% of bound nucleic acid is eluted in the standard elution volume at a high concentration.
- **Convenient elution:** For convenience, elution buffer of ambient temperature may be used. This will result in a lower yield (approximately 20 %) compared to elution with preheated elution buffer.

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and / or multi use storage at 4°C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications.

For optimal performance of isolated DNA in subsequent downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Performance of long-range PCR (e.g., > 10 kbp) or detection sensitivity of trace amount of DNA species might be reduced after multiple freeze-thaw cycles or prolonged storage of eluted DNA at +4 °C or room temperature due to DNA shearing or adsorption to surfaces.

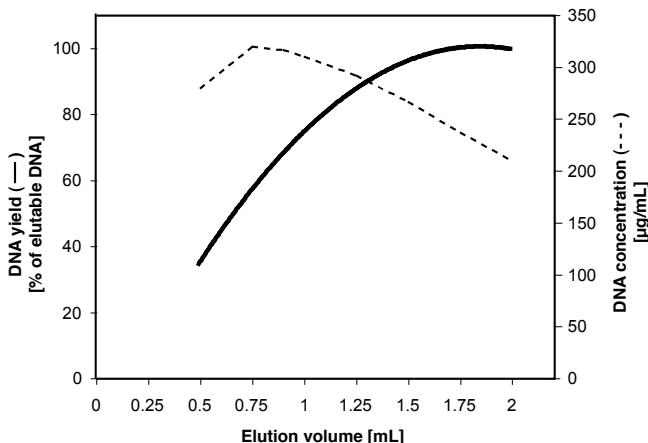


Figure 1: Dependence of DNA yield (solid line) and concentration (dashed line) on elution volume.

Genomic DNA was purified from 10 mL whole blood and eluted using different elution volumes as indicated. Highest DNA yield is obtained with 1.5-2.0 mL elution volume. Highest DNA concentration is obtained with approximately 0.75 mL elution volume. Furthermore, yield and concentration may vary as they depend on the kind of sample (blood, serum, plasma), type of blood sample (human or animal), and quality of the samples (fresh, old, frozen, clotted, etc.).

3 Storage conditions and preparation of working solutions

Attention:

Buffers B3, BQ1, and BW contain chaotropic salt. Wear gloves and goggles!

CAUTION: Buffer B3, BQ1, and BW contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- All kit components can be stored at room temperature (18–25 °C) and are stable up to one year.
- During storage, especially at low temperatures, a white precipitate may form in Buffer T1, B3, or BQ1. Such precipitates can be easily dissolved by incubating the bottle at 70°C before use.

Before starting any **NucleoSpin® Blood** protocol prepare the following:

- **Wash Buffer B5** (NucleoSpin® Blood): Add the indicated volume of ethanol (96–100 %) to **Wash Buffer B5 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer B5 at room temperature (18–25 °C) for up to one year.
- **Wash Buffer BQ2** (NucleoSpin® Blood L/XL/QuickPure): Add the indicated volume of ethanol (96–100 %) to **Wash Buffer BQ2 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer BQ2 at room temperature (18–25 °C) for up to one year.
- **Proteinase K**: Add the indicated volume of Proteinase Buffer PB to dissolve lyophilized **Proteinase K**. Proteinase K solution is stable at -20 °C for up to 6 months.

| NucleoSpin® Blood | | | |
|---------------------------------|---|---|---|
| REF | 10 preps | 50 preps | 250 preps |
| | 740951.10 | 740951.50 | 740951.250 |
| Wash Buffer B5 (Concentrate) | 6 mL Add 24 mL ethanol | 12 mL Add 48 mL ethanol | 50 mL Add 200 mL ethanol |
| Proteinase K | 6 mg Add 260 µL Proteinase Buffer | 30 mg Add 1.35 mL Proteinase Buffer | 2 x 75 mg Add 3.35 mL Proteinase Buffer to each vial |

| NucleoSpin® Blood L | | | |
|----------------------------------|---|--|--|
| REF | NucleoSpin® Blood L | NucleoSpin® Blood XL | NucleoSpin® Blood XL |
| | 20 preps | 10 preps | 50 preps |
| Wash Buffer BQ2 (Concentrate) | 20 mL Add 80 mL ethanol | 50 mL Add 200 mL ethanol | 4 x 50 mL Add 200 mL ethanol to each bottle |
| Proteinase K | 60 mg Add 3.15 mL Proteinase Buffer | 126 mg Add 5.75 mL Proteinase Buffer | 5 x 126 mg Add 5.75 mL Proteinase Buffer to each vial |

| NucleoSpin® Blood QuickPure | | | |
|------------------------------------|---|---|---|
| REF | 10 preps | 50 preps | 250 preps |
| | 740569.10 | 740569.50 | 740569.250 |
| Wash Buffer BQ2 (Concentrate) | 7 mL Add 28 mL ethanol | 7 mL Add 28 mL ethanol | 2 x 20 mL Add 80 mL ethanol to each bottle |
| Proteinase K | 6 mg Add 260 µL Proteinase Buffer | 30 mg Add 1.35 mL Proteinase Buffer | 2 x 75 mg Add 3.35 mL Proteinase Buffer to each vial |

4 Safety instructions

The following components of the **NucleoSpin® Blood** kits contain hazardous contents.
Wear gloves and goggles and follow the safety instructions given in this section.

GHS classification

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Mindergefährliche Eigenschaften müssen bis 125 mL oder 125 g nicht mit H- und P-Sätzen gekennzeichnet werden.

| Component | Hazard contents | GHS symbol | Hazard phrases | Precaution phrases |
|--------------|--|---|--------------------|---|
| Inhalt | Gefahrstoff | GHS Symbol | H-Sätze | P-Sätze |
| B3 | Guanidine hydrochloride 36–50 % <i>Guanidinhydrochlorid 36–50 %</i> | | Warning Achtung | 302, 319 280, 301+312, 305+351+338, 330, 337+313 |
| BQ1 | Guanidine hydrochloride 50–66 % <i>Guanidinhydrochlorid 50–66 %</i> | | Warning Achtung | 302, 315, 319 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313 |
| BW | Guanidine hydrochloride 36–50 % + isopropanol 20–50 % <i>Guanidinhydrochlorid 36–50 % + Isopropanol 20–50 %</i> |  | Warning Achtung | 226, 302, 319 210, 233, 280, 301+312, 305+351+338, 330, 337+313, 403+235 |
| Proteinase K | Proteinase K, lyophilized <i>Proteinase K, lyophilisiert</i> |  | Danger Gefahr | 315, 319, 334, 335 261, 280, 302+352, 304+340, 305+351+338, 312, 332+313, 337+313, 342+311, 403+233 |

Hazard phrases

- H 226 Flammable liquid and vapour.
Flüssigkeit und Dampf entzündbar.
- H 302 Harmful if swallowed.
Gesundheitsschädlich bei Verschlucken.
- H 315 Causes skin irritation.
Verursacht Hautreizungen.
- H 317 May cause an allergic skin reaction.
Kann allergische Hautreaktionen verursachen.
- H 319 Causes serious eye irritation.
Verursacht schwere Augenreizung.
- H 334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Kann bei Einatmen Allergie, asthmatische Symptome oder Atembeschwerden verursachen.
- H 335 May cause respiratory irritation.
Kann die Atemwege reizen.

Precaution phrases

- P 210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Von Hitze, heißen Oberflächen, Funken, offenen Flammen sowie anderen Zündquellenarten fernhalten. Nicht rauchen.
- P 233 Keep container tightly closed.
Behälter dicht verschlossen halten.
- P 261 Avoid breathing dust.
Einatmen von Staub vermeiden.
- P 280 Wear protective gloves / eye protection.
Schutzhandschuhe/Augenschutz tragen.
- P 301+312 IF SWALLOWED: Call a POISON CENTER/ doctor/.../if you feel unwell.
BEI VERSCHLUCKEN: Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt/... anrufen.
- P 302+352 IF ON SKIN: Wash with plenty of water/...
BEI KONTAKT MIT DER HAUT: Mit viel Wasser und Seife waschen.
- P 304+340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
BEI EINATMEN: An die frische Luft bringen und in einer Position ruhigstellen, die das Atmen erleichtert.
- P 305+351+338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
BEI KONTAKT MIT DEN AUGEN: Einige Minuten lang behutsam mit Wasser spülen. Vorhandene Kontaktlinsen nach Möglichkeit entfernen. Weiter spülen.
- P 312 Call a POISON CENTER/ doctor/.../if you feel unwell.
Bei Unwohlsein GIFTINFORMATIONSZENTRUM / Arzt/... anrufen.
- P 330 Rinse mouth.
Mund ausspülen.
- P 332+313 If skin irritation occurs: Get medical advice / attention.
Bei Hautreizung: Ärztlischen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 337+313 Get medical advice / attention.
Bei anhaltende Augenreizung: Ärztlischen Rat einholen / ärztliche Hilfe hinzuziehen.
- P 342+311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor/...
Bei Symptomen der Atemwege: GIFTINFORMATIONSZENTRUM / Arzt/... anrufen.
- P 403+233 Store in a well ventilated place. Keep container tightly closed.
Behälter dicht verschlossen an einem gut belüfteten Ort aufbewahren.
- P 403+235 Store in a well ventilated place. Keep cool.
Kühl an einem gut belüfteten Ort aufbewahren.

For further information please see Material Safety Data Sheets (www.mn-net.com).
Weiterführende Informationen finden Sie in den Sicherheitsdatenblättern (www.mn-net.com).

5 Protocols for DNA purification from whole blood

5.1 Genomic DNA purification with NucleoSpin® Blood

Before starting the preparation:

- Check if Buffer B5 and Proteinase K were prepared according to section 3.
 - Set an incubator or water bath to 70 °C.
 - Preheat Elution Buffer BE to 70 °C.
-

1 Lyse blood sample

Pipette **25 µL Proteinase K** and up to **200 µL blood**, body fluid sample, or buffy coat from 1 mL blood (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided).

For sample volumes less than 200 µL, add PBS to adjust the volume to 200 µL. If purifying DNA viruses, we recommend starting with 200 µL serum or plasma. If cultured cells are used, resuspend up to 5×10^6 cells in a final volume of 200 µL PBS.

Add **200 µL Buffer B3** to the samples and vortex the mixture vigorously (10–20 s).

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

Incubate samples at **70 °C** for **10–15 min**.

The lysate should become brownish during incubation with Buffer B3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **210 µL ethanol (96–100 %)** to each sample and vortex again.

200 µL blood

**+ 25 µL
Proteinase K**



+ 200 µL B3

Mix

**70 °C
10–15 min**



**+ 210 µL
ethanol**

Mix

3 Bind DNA

For each preparation, take one NucleoSpin® Blood Column placed in a Collection Tube and load the sample. Centrifuge 1 min at 11,000 x g. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (< 15,000 x g). Discard Collection Tube with flow-through.



Load lysate



11,000 x g
1 min

4 Wash silica membrane

1st wash

Place the NucleoSpin® Blood Column into a new Collection Tube (2 mL) and add 500 µL Buffer BW. Centrifuge 1 min at 11,000 x g. Discard Collection Tube with flow-through.



+ 500 µL BW



11,000 x g
1 min

2nd wash

Place the NucleoSpin® Blood Column into a new Collection Tube (2 mL) and add 600 µL Buffer B5. Centrifuge 1 min at 11,000 x g. Discard flow-through and reuse Collection Tube.



+ 600 µL B5



11,000 x g
1 min

5 Dry silica membrane

Place the NucleoSpin® Blood Column back into the Collection Tube and centrifuge 1 min at 11,000 x g.



11,000 x g
1 min



Residual ethanol is removed during this step.

6 Elute highly pure DNA

Place the NucleoSpin® Blood Column in a 1.5 mL microcentrifuge tube (not provided) and add 100 µL preheated Buffer BE (70 °C). Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.



+ 100 µL BE
(70 °C)



RT
1 min

For alternative elution procedures see section 2.4.



11,000 x g
1 min

5.2 Genomic DNA purification with NucleoSpin® Blood L

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
 - Set an incubator or water bath to 56 °C.
 - Preheat Elution Buffer BE to 70 °C.
 - For centrifugation, a centrifuge with a **swing-out rotor** and appropriate buckets capable of reaching 4,000–4,500 x g is required.
-

1 Lyse blood sample

Pipette up to **2 mL** **blood** (or body fluid) sample (equilibrated to room temperature) and **150 µL** **Proteinase K** into a 15 mL tube (not provided).

If processing buffy coat, do not use more than 1 mL and add PBS to adjust the volume to 2 mL.

If cultured cells are used, resuspend up to 2×10^7 cells in a final volume of 2 mL PBS.

Add **2 mL** **Buffer BQ1** (if processing less than 2 mL blood, add one volume of Buffer BQ1) to the samples and vortex the mixture vigorously for 10 s.

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

Incubate samples at **56 °C** for **15 min**.

Let the samples cool down to room temperature before proceeding with addition of ethanol.

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 20 min) and vortex once or twice during incubation if processing older or clotted blood samples.

2 mL blood

+ 150 µL
Proteinase K



+ 2 mL BQ1

Mix

56 °C

15 min

2 Adjust DNA binding conditions

Add **2 mL ethanol (96–100 %)** (if processing less than 2 mL blood, add 1 volume of ethanol) to each sample and mix by inverting the tube 10 times.

+ 2 mL
ethanol

Note: High local ethanol concentration must be avoided by immediate mixing after addition.

Mix

Be sure that the lysate has cooled down to room temperature before loading it onto the column. Loading of hot lysate may lead to diminished yields.

3 Bind DNA

For each preparation, take one **NucleoSpin® Blood L Column** placed in a Collection Tube and **load 3 mL of lysate**. Do not moisten the rims of the columns. Close the tubes with screw caps and centrifuge **3 min at 4,500 x g**.

Load 3 mL



4,500 x g
3 min

Usually the lysate will start to flow-through the columns even before centrifugation. This will not adversely affect DNA yield or purity. Keep NucleoSpin® Blood L Column in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.

Load **all of the remaining lysate** in a second step to the respective NucleoSpin® Blood L Column, avoiding moistening the rim. Centrifuge **5 min at 4,500 x g**. Discard the flow-through and place the column back into the Collection Tube.

Load residue



4,500 x g
5 min

Remove the Collection Tube with the column carefully from the rotor to avoid that the flow-through comes in contact with the column outlet. Be sure to wipe off any spilled lysate from the Collection Tube before placing the column back.

4 Wash silica membrane

1st wash

Add **2 mL Buffer BQ2**. Centrifuge **2 min** at **4,500 x g**.

It is not necessary to discard the flow-through after the first washing step.

+ **2 mL BQ2**

4,500 x g
2 min

**2nd wash**

Add **2 mL Buffer BQ2**. Centrifuge **10 min** at **4,500 x g**. Remove the column carefully from the rotor in order to avoid that the flow-through comes in contact with the column outlet.

+ **2 mL BQ2**

4,500 x g
10 min



By prolonged centrifugation during this second washing step, residual ethanolic washing Buffer BQ2 is removed from the silica membrane of the NucleoSpin® Blood L Column.

5 Dry silica membrane

The drying of the NucleoSpin® Blood L Column is performed by prolonged centrifugation time (10 min) in the 2nd wash step.

6 Elute highly pure DNA

Insert the column into a new Collection Tube (15 mL) and apply **200 µL preheated Buffer BE (70 °C)** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Centrifuge at **4,500 x g** for **2 min**.

For alternative elution procedures see section 2.4.

+ **200 µL BE**
(70 °C)

RT
1 min



4,500 x g
2 min



5.3 Genomic DNA purification with NucleoSpin® Blood XL

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 56 °C.
- Preheat Elution Buffer BE to 70 °C.
- For centrifugation, a centrifuge with a **swing-out rotor** and appropriate buckets capable of reaching 4,000–4,500 $\times g$ is required.

1 Lyse blood sample

Pipette up to **10 mL blood** (or body fluid) sample (equilibrated to room temperature) and **500 µL Proteinase K** into a 50 mL tube (not provided).

If you process ≤ 5 mL blood, sample loading with a single centrifugation step is possible (step 3).

If processing buffy coat, do not use more than 2 mL and add PBS to adjust the volume to 10 mL.

If cultured cells are used, resuspend up to 1×10^8 cells in a final volume of 10 mL PBS.

Add **10 mL Buffer BQ1** (if processing less than 10 mL blood, add one volume of Buffer BQ1) to the samples and vortex the mixture vigorously for 10 s.

Note: *Vigorous mixing is important to obtain high yield and purity of DNA.*

Incubate samples at **56 °C** for **15 min**.

Let the lysate cool down to room temperature before proceeding with addition of ethanol.

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 20 min) and vortex once or twice during incubation if processing older or clotted blood samples.

10 mL blood

**+ 500 µL
Proteinase K**

+ 10 mL BQ1

Mix



**56 °C
15 min**

2 Adjust DNA binding conditions

Add **10 mL ethanol (96–100 %)** (if processing less than 10 mL blood, add one volume of ethanol) to each sample and mix by inverting the tube 10 times.

Note: High local ethanol concentration must be avoided by immediate mixing after addition.

Be sure that the lysate has cooled down to room temperature (about 5 min) before loading it onto the columns. Loading of hot lysate may lead to diminished yields.

+ 10 mL
ethanol

Mix

3 Bind DNA

For each preparation, take one **NucleoSpin® Blood XL Column** placed in a Collection Tube and load **15 mL of lysate**. Do not moisten the rim of the column. Close the tubes with screw caps and centrifuge **3 min at 4,000 x g**. Discard flow-through.

Discarding the flow-through may be omitted. Be careful after the second loading step during removal of the tube from the centrifuge and the removal of the column from the tube: keep tube with column upright to avoid contact of flow-through with the column outlet.

Usually the lysate will start to flow through the column even before centrifugation. This will not adversely affect DNA yield or purity. Keep NucleoSpin® Blood XL Column in an upright position as liquid may pass through the ventilation slots on the rim of the column even if the caps are closed.

Load **15 mL of the remaining lysate** to the respective NucleoSpin® Blood XL Column. Again, avoid moistening the rim. Centrifuge **3 min at 4,000 x g**. Discard the flow-through and place the column back into the Collection Tube.

Remove the Collection Tube with the column carefully from the rotor and avoid that the flow-through comes in contact with the column outlet.

If you process ≤ 5 mL blood no loading of remaining lysate is necessary.

Load **15 mL**



4,000 x g
3 min

Load residue



4,000 x g
3 min

4 Wash silica membrane

1st wash

Add **7.5 mL Buffer BQ2** to the NucleoSpin® Blood XL Column. Centrifuge **2 min** at **4,000 x g**.

It is not necessary to discard the flow-through after the first washing step.

+ 7.5 mL BQ2**4,000 x g**
2 min**2nd wash**

Add **7.5 mL Buffer BQ2**. Centrifuge **10 min** at **4,000 x g**. Remove the column carefully from the rotor to avoid that flow-through gets in contact with the column outlet.

By prolonged centrifugation during this second washing step, residual ethanolic Buffer BQ2 is removed from the silica membrane of the NucleoSpin® Blood XL Column.

**+ 7.5 mL BQ2****4,000 x g**
10 min

5 Dry silica membrane

The drying of the NucleoSpin® Blood XL Column is performed by prolonged centrifugation time (10 min) in the 2nd wash step.

6 Elute highly pure DNA

Insert the column into a new Collection Tube (50 mL) and apply **1000 µL of preheated Buffer BE (70 °C)** directly to the center of the silica membrane. Incubate at **room temperature** for **2 min**. Centrifuge at **4,000 x g** for **2 min**.

For alternative elution procedures see section 2.4.

**+ 1000 µL BE
(70 °C)****RT**
2 min**4,000 x g**
2 min

5.4 Genomic DNA purification with NucleoSpin® Blood QuickPure

Before starting the preparation:

- Check if Buffer BQ2 and Proteinase K were prepared according to section 3.
- Set an incubator or water bath to 70 °C.
- Preheat Elution Buffer BE to 70 °C.

1 Lyse blood sample

Pipette **25 µL Proteinase K** and up to **200 µL blood**, buffy coat or body fluid sample (equilibrated to room temperature) into 1.5 mL microcentrifuge tubes (not provided).

200 µL blood

For sample volumes less than 200 µL, add PBS to adjust the volume to 200 µL. If cultured cells are used, resuspend up to 5×10^6 cells in a final volume of 200 µL PBS.

+ 25 µL Proteinase K

Add **200 µL Lysis Buffer BQ1** to the samples and vortex the mixture vigorously (10–20 s).

+ 200 µL BQ1

Note: Vigorous mixing is important to obtain high yield and purity of DNA.

Mix

Incubate samples at **70 °C** for **10–15 min**.

70 °C
10–15 min

The lysate should become brownish during incubation with Buffer BQ1. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples.

2 Adjust DNA binding conditions

Add **200 µL ethanol (96–100 %)** to each sample and vortex again.

+ 200 µL ethanol

Mix

3 Bind DNA

Apply the samples to the **NucleoSpin® Blood QuickPure Columns** placed in a Collection Tube and centrifuge **1 min at 11,000 x g**. If the samples are not drawn through the matrix completely, repeat the centrifugation at higher g-force (up to 15,000 x g). Discard Collection Tube with flow-through.

Load lysate

**11,000 x g
1 min**

4 Wash silica membrane

Place the NucleoSpin® Blood QuickPure Column into a new Collection Tube (2 mL) and add **350 µL Buffer BQ2**. Centrifuge **3 min at 11,000 x g**. Discard Collection Tube with flow-through.

Optional: Place the NucleoSpin® Blood QuickPure Column into a new Collection Tube (2 mL; not provided) and add 200 µL Buffer BQ2. Centrifuge 1 min at 11,000 x g. Discard flow-through and Collection Tube and proceed to step 6.

This additional washing step is only recommended if the DNA is intended for use as a template in especially critical PCRs. In the vast majority of cases, you can save time by this step.



+ 350 µL BQ2



11,000 x g
3 min

5 Dry silica membrane

The drying of the NucleoSpin® Blood QuickPure Column is performed by the 3 min centrifugation in step 4.

6 Elute highly pure DNA

Place the NucleoSpin® Blood QuickPure Column in a 1.5 mL microcentrifuge tube (not provided) and add **50 µL prewarmed Buffer BE (70 °C)**. Dispense buffer directly onto the silica membrane. Incubate at **room temperature** for **1 min**. Centrifuge **1 min at 11,000 x g**.



+ 50 µL BE
(70 °C)



RT
1 min

For alternative elution procedures see section 2.4.



11,000 x g
1 min

6 Appendix

6.1 Troubleshooting

| Problem | Possible cause and suggestions |
|----------------------|--|
| | <p><i>Low concentration of leukocytes in sample</i></p> <ul style="list-style-type: none">• Prepare buffy coat from the blood sample: Centrifuge whole blood at room temperature ($3,300 \times g$; 10 min). Three different layers will be visible after centrifugation. Leukocytes are concentrated in the intermediate layer (= buffy coat). |
| | <p><i>Incomplete cell lysis</i></p> <ul style="list-style-type: none">• Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer.• Proteinase K digestion is not optimal. Never add Proteinase K directly to lysis buffer. Incubate for 15–20 min at 70°C / 56°C. |
| No or poor DNA yield | <p><i>Reagents not applied properly</i></p> <ul style="list-style-type: none">• Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysates before loading them on columns. |
| | <p><i>Suboptimal elution of DNA from the column</i></p> <ul style="list-style-type: none">• Preheat Buffer BE to 70°C before elution. Apply Buffer BE directly onto the center of the silica membrane.• Elution efficiencies decrease dramatically if elution is performed with buffers of $\text{pH} < 7.0$. Use slightly alkaline elution buffer like Buffer BE ($\text{pH } 8.5$).• Mix vigorously once during the 70°C / 56°C incubation step especially when working with older or clotted blood samples. |
| Poor DNA quality | <p><i>Reagents not applied properly</i></p> <ul style="list-style-type: none">• Prepare buffers and Proteinase K solution according to instructions (section 3). Add ethanol to lysates and mix before loading them on columns. <p><i>Incomplete cell lysis</i></p> <ul style="list-style-type: none">• Sample not thoroughly mixed with lysis buffer / Proteinase K. The mixture has to be vortexed vigorously immediately after addition of lysis buffer. |

- Proteinase K digestion is not optimal. Do not add Proteinase K directly to lysis buffer. Incubate for at least 15–20 min at 56 °C / 70 °C.

RNA in sample

- If RNA-free DNA is desired, add 20 µL RNase A solution (20 mg/mL) before addition of lysis buffer.

Old or clotted blood samples processed

- For isolation of DNA from older or clotted blood samples, we recommend prolonging Proteinase K incubation to 30 min and vortexing several times during this step. Especially for NucleoSpin® Blood L/XL with troublesome blood samples performance can be improved by the following steps:

First incubate the lysate for 10–15 min at room temperature. Incubate for 15 min at the recommended 56 °C afterwards. Clear lysate before addition of ethanol. It is recommended performing a short centrifugation step of about 30–60 s after the lysis of the sample material (before addition of ethanol) in order to pellet non-lysed clumps.

In case of difficult blood samples it might happen that the washing steps with ethanolic Buffer BQ2 are not sufficient to remove all contamination. An additional wash step with a buffer including chaotropic salt is recommended, for example water/BQ1/ethanol mix (1:1:1). Afterwards, the washing step with ethanolic Buffer BQ2 should be performed to completely remove the chaotropic salt of the wash buffer.

Poor DNA quality
(continued)

Suboptimal performance of genomic DNA in enzymatic reactions

Carry-over of ethanol

- Be sure to remove all of ethanolic Buffer B5 / BQ2 before eluting the DNA. If the level of B5 / BQ2 after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the Collection Tube, and centrifuge again.

Suboptimal performance of genomic DNA in enzymatic reactions
(continued)

Contamination of DNA with inhibitory substances

- If DNA has been eluted with Tris/EDTA-buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in Buffer BE.
- If preparing DNA from older or clotted blood samples, extend Proteinase K incubation to 30 min and vortex once or twice during this step.
- If the A_{260}/A_{280} ratio of the eluate is below 1.6, repeat the purification procedure:

For **NucleoSpin® Blood**: Add 1 volume of Buffer B3 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood Column, and proceed with step 3 of the corresponding protocol.

For **NucleoSpin® Blood QuickPure**: Add 1 volume of Buffer BQ1 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood QuickPure Column, and proceed with step 3 of the corresponding protocol

For **NucleoSpin® Blood L / XL**: Add 1 volume of Buffer BQ1 plus 1 volume ethanol to the eluate, load on NucleoSpin® Blood L/XL Column, and proceed with step 3 of the corresponding protocol.

6.2 Ordering information

| Product | REF | Pack of |
|---|---------------------|-----------------|
| NucleoSpin® Blood | 740951.10/.50/.250 | 10/50/250 |
| NucleoSpin® Blood L | 740954.20 | 20 |
| NucleoSpin® Blood XL | 740950.10/.50 | 10/50 |
| NucleoSpin® Blood QuickPure | 740569.10/.50/.250 | 10/50/250 |
| Buffer BQ1 | 740923 | 125 mL |
| Buffer B3 | 740920 | 100 mL |
| Buffer B5 Concentrate (for 100 mL Buffer B5) | 740921 | 20 mL |
| Bufer BW | 740922 | 100 mL |
| Proteinase K | 740506 | 100 mg |
| RNase A | 740505.50 740505 | 50 mg 100 mg |
| Collection Tubes (2 mL) | 740600 | 1000 |

Visit www.mn-net.com for more detailed product information.

6.3 Reference

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

6.4 Product use restriction/warranty

NucleoSpin® Blood kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN-VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN-VITRO*-diagnostic use. Please pay attention to the package of the product. *IN-VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN-VITRO*-DIAGNOSTIC USE!

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No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or - in any case of resale of the products - in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

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